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Search History**Today's Date: 8/8/2001**

<u>DB Name</u>	<u>Query</u>	<u>Hit Count</u>	<u>Set Name</u>
USPT	115 not (phenol or chloroform)	95	<u>L16</u>
USPT	112 and (NaCl same Triton same extract\$) and (amplif\$ same rna)	183	<u>L15</u>
USPT	(NaCl same Triton same extract\$) and (amplif\$ same rna)	194	<u>L14</u>
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USPT	(non-ionic same soluble) and (rna same extract\$)	55	<u>L3</u>
USPT	11 and (non-ionic same soluble)	8	<u>L2</u>
USPT	(amplif\$ or pcr) same (primer\$1 or probe\$1) same rna same extract\$	1541	<u>L1</u>

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Search Results - Record(s) 1 through 10 of 95 returned.

☐ 1. Document ID: US 6261765 B1

L16: Entry 1 of 95

File: USPT

Jul 17, 2001

US-PAT-NO: 6261765

DOCUMENT-IDENTIFIER: US 6261765 B1

TITLE: In vitro method for disassembly/reassembly of papillomavirus virus-like particles (VLPs)

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Draw Desc	Image
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☐ 2. Document ID: US 6258584 B1

L16: Entry 2 of 95

File: USPT

Jul 10, 2001

US-PAT-NO: 6258584

DOCUMENT-IDENTIFIER: US 6258584 B1

TITLE: Recombinant C-proteinase and processes, methods and uses thereof

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Draw Desc	Image
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☐ 3. Document ID: US 6255281 B1

L16: Entry 3 of 95

File: USPT

Jul 3, 2001

US-PAT-NO: 6255281

DOCUMENT-IDENTIFIER: US 6255281 B1

TITLE: Use of recombinant human uteroglobin in treatment of inflammatory and fibrotic conditions

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 4. Document ID: US 6245905 B1

L16: Entry 4 of 95

File: USPT

Jun 12, 2001

US-PAT-NO: 6245905

DOCUMENT-IDENTIFIER: US 6245905 B1

TITLE: Nucleic acid molecule encoding abscisic acid responsive element-binding factor 2

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 5. Document ID: US 6242668 B1

L16: Entry 5 of 95

File: USPT

Jun 5, 2001

US-PAT-NO: 6242668

DOCUMENT-IDENTIFIER: US 6242668 B1

TITLE: Strawberry endo-1,4-.beta.-glucanase genes and their uses

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 6. Document ID: US 6232461 B1

L16: Entry 6 of 95

File: USPT

May 15, 2001

US-PAT-NO: 6232461

DOCUMENT-IDENTIFIER: US 6232461 B1

TITLE: Nucleic acid molecule encoding abscisic acid responsive element-binding ractor 4

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 7. Document ID: US 6232070 B1

L16: Entry 7 of 95

File: USPT

May 15, 2001

US-PAT-NO: 6232070

DOCUMENT-IDENTIFIER: US 6232070 B1

TITLE: Pharmacological targeting of mRNA cap formation

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 8. Document ID: US 6218527 B1

L16: Entry 8 of 95

File: USPT

Apr 17, 2001

US-PAT-NO: 6218527

DOCUMENT-IDENTIFIER: US 6218527 B1

TITLE: Nucleic acid molecule encoding abscisic acid responsive element-binding factor 3

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 9. Document ID: US 6214794 B1

L16: Entry 9 of 95

File: USPT

Apr 10, 2001

DOCUMENT-IDENTIFIER: US 6214794 B1
TITLE: Method of using hedgehog polypeptides to regulate neuronal cell growth

KMNC	Drawn Desc	Image
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Apr 3, 2001

TITLE: Recombinant .alpha.-2,3-sialyltransferases and their uses

KMJC	Drawn Desc	Image
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☐ 11. Document ID: US 6207816 B1

L16: Entry 11 of 95

File: USPT

Mar 27, 2001

US-PAT-NO: 6207816

DOCUMENT-IDENTIFIER: US 6207816 B1

TITLE: High affinity oligonucleotide ligands to growth factors

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 12. Document ID: US 6194559 B1

L16: Entry 12 of 95

File: USPT

Feb 27, 2001

US-PAT-NO: 6194559

DOCUMENT-IDENTIFIER: US 6194559 B1

TITLE: Abscissic acid responsive element-binding transcription factors

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 13. Document ID: US 6187559 B1

L16: Entry 13 of 95

File: USPT

Feb 13, 2001

US-PAT-NO: 6187559

DOCUMENT-IDENTIFIER: US 6187559 B1

TITLE: Phospholipase D gene

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 14. Document ID: US 6153395 A

L16: Entry 14 of 95

File: USPT

Nov 28, 2000

US-PAT-NO: 6153395

DOCUMENT-IDENTIFIER: US 6153395 A

TITLE: ICAM-related protein

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 15. Document ID: US 6150508 A

L16: Entry 15 of 95

File: USPT

Nov 21, 2000

US-PAT-NO: 6150508

DOCUMENT-IDENTIFIER: US 6150508 A

TITLE: Monoclonal antibodies specific for the extracellular domain of prostate-specific membrane antigen

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 16. Document ID: US 6146849 A

L16: Entry 16 of 95

File: USPT

Nov 14, 2000

US-PAT-NO: 6146849

DOCUMENT-IDENTIFIER: US 6146849 A

TITLE: Lectins and coding sequences

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 17. Document ID: US 6133434 A

L16: Entry 17 of 95

File: USPT

Oct 17, 2000

US-PAT-NO: 6133434

DOCUMENT-IDENTIFIER: US 6133434 A

TITLE: Purinergic receptor

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 18. Document ID: US 6132728 A

L16: Entry 18 of 95

File: USPT

Oct 17, 2000

US-PAT-NO: 6132728

DOCUMENT-IDENTIFIER: US 6132728 A

TITLE: Hedgehog-derived polypeptides

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 19. Document ID: US 6123938 A

L16: Entry 19 of 95

File: USPT

Sep 26, 2000

US-PAT-NO: 6123938

DOCUMENT-IDENTIFIER: US 6123938 A

TITLE: Human urinary hyaluronidase

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KVMC	Drawl Desc	Image
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☐ 20. Document ID: US 6124091 A

L16: Entry 20 of 95

File: USPT

Sep 26, 2000

US-PAT-NO: 6124091

DOCUMENT-IDENTIFIER: US 6124091 A

TITLE: Cell growth-controlling oligonucleotides

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KVMC	Drawl Desc	Image
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(15 NOT (CHOLOROFORM OR PHENOL)).USPT.	95

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Search Results - Record(s) 31 through 40 of 95 returned.

☐ 31. Document ID: US 6057091 A

L16: Entry 31 of 95

File: USPT

May 2, 2000

US-PAT-NO: 6057091

DOCUMENT-IDENTIFIER: US 6057091 A

TITLE: Method of identifying compounds affecting hedgehog cholesterol transfer

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 32. Document ID: US 6051226 A

L16: Entry 32 of 95

File: USPT

Apr 18, 2000

US-PAT-NO: 6051226

DOCUMENT-IDENTIFIER: US 6051226 A

TITLE: MN-specific antibodies and their use in cancer treatment

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 33. Document ID: US 6046307 A

L16: Entry 33 of 95

File: USPT

Apr 4, 2000

US-PAT-NO: 6046307

DOCUMENT-IDENTIFIER: US 6046307 A

TITLE: Modulation of mammalian telomerase by peptide nucleic acids

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 34. Document ID: US 6046384 A

L16: Entry 34 of 95

File: USPT

Apr 4, 2000

US-PAT-NO: 6046384

DOCUMENT-IDENTIFIER: US 6046384 A

TITLE: Papaya ringspot virus NIa protease gene

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 35. Document ID: US 6043083 A

L16: Entry 35 of 95

File: USPT

Mar 28, 2000

US-PAT-NO: 6043083

DOCUMENT-IDENTIFIER: US 6043083 A

TITLE: Inhibitors of the JNK signal transduction pathway and methods of use

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 36. Document ID: US 6030785 A

L16: Entry 36 of 95

File: USPT

Feb 29, 2000

US-PAT-NO: 6030785

DOCUMENT-IDENTIFIER: US 6030785 A

TITLE: Screening methods to identify agents that selectively inhibit hepatitis C virus replication

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 37. Document ID: US 6025183 A

L16: Entry 37 of 95

File: USPT

Feb 15, 2000

US-PAT-NO: 6025183

DOCUMENT-IDENTIFIER: US 6025183 A

TITLE: Transgenic animal assay system for anti-cholinesterase substances

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 38. Document ID: US 6020193 A

L16: Entry 38 of 95

File: USPT

Feb 1, 2000

US-PAT-NO: 6020193

DOCUMENT-IDENTIFIER: US 6020193 A

TITLE: Recombinant C-proteinase and processes, methods and uses thereof

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 39. Document ID: US 6015700 A

L16: Entry 39 of 95

File: USPT

Jan 18, 2000

US-PAT-NO: 6015700

DOCUMENT-IDENTIFIER: US 6015700 A

TITLE: Cdc2 protein kinase from pneumocystis carinii

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 40. Document ID: US 6015710 A

L16: Entry 40 of 95

File: USPT

Jan 18, 2000

US-PAT-NO: 6015710

DOCUMENT-IDENTIFIER: US 6015710 A

TITLE: Modulation of mammalian telomerase by peptide nucleic acids

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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Search Results - Record(s) 41 through 50 of 95 returned.

☐ 41. Document ID: US 6013782 A

L16: Entry 41 of 95

File: USPT

Jan 11, 2000

US-PAT-NO: 6013782

DOCUMENT-IDENTIFIER: US 6013782 A

TITLE: Integrin-linked kinase and its uses

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 42. Document ID: US 6004535 A

L16: Entry 42 of 95

File: USPT

Dec 21, 1999

US-PAT-NO: 6004535

DOCUMENT-IDENTIFIER: US 6004535 A

TITLE: Methods of imaging neoplastic disease and of detecting and quantifying MN protein/polypeptide using MN-specific antibodies

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 43. Document ID: US 6004793 A

L16: Entry 43 of 95

File: USPT

Dec 21, 1999

US-PAT-NO: 6004793

DOCUMENT-IDENTIFIER: US 6004793 A

TITLE: Method for cloning and producing the *Ava*I restriction endonuclease in *E. coli* and purification of the recombinant *Ava*I restriction endonuclease

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 44. Document ID: US 6002072 A

L16: Entry 44 of 95

File: USPT

Dec 14, 1999

US-PAT-NO: 6002072

DOCUMENT-IDENTIFIER: US 6002072 A

TITLE: Coat protein gene for the FLA83 W strain of papaya ringspot virus

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 45. Document ID: US 5981831 A

L16: Entry 45 of 95

File: USPT

Nov 9, 1999

US-PAT-NO: 5981831

DOCUMENT-IDENTIFIER: US 5981831 A

TITLE: Exo-(1--4)-.beta.-D galactanase

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 46. Document ID: US 5977442 A

L16: Entry 46 of 95

File: USPT

Nov 2, 1999

US-PAT-NO: 5977442

DOCUMENT-IDENTIFIER: US 5977442 A

TITLE: Salicylic acid induced map kinase and its use for enhanced disease resistance in plants

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 47. Document ID: US 5972605 A

L16: Entry 47 of 95

File: USPT

Oct 26, 1999

US-PAT-NO: 5972605

DOCUMENT-IDENTIFIER: US 5972605 A

TITLE: Assays for regulators of mammalian telomerase expression

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 48. Document ID: US 5968761 A

L16: Entry 48 of 95

File: USPT

Oct 19, 1999

US-PAT-NO: 5968761

DOCUMENT-IDENTIFIER: US 5968761 A

TITLE: Ubiquitin conjugating enzymes

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 49. Document ID: US 5962404 A

L16: Entry 49 of 95

File: USPT

Oct 5, 1999

US-PAT-NO: 5962404
DOCUMENT-IDENTIFIER: US 5962404 A
TITLE: Enzymatically-produced oligodendrocyte cytotoxic dimeric IL-2 factor

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 50. Document ID: US 5948643 A

L16: Entry 50 of 95

File: USPT

Sep 7, 1999

US-PAT-NO: 5948643
DOCUMENT-IDENTIFIER: US 5948643 A
TITLE: Modulators of BRCA1 activity

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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PHENOL.USPT.	109596
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CHOLOROFORMS	0
(15 NOT (CHOLOROFORM OR PHENOL)).USPT.	95

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Documents, starting with Document:

51

Display Format: TI

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L16: Entry 52 of 95

File: USPT

Jun 1, 1999

DOCUMENT-IDENTIFIER: US 5908750 A

TITLE: Screening assays for identifying agents that regulate the expression of genes involved in cell death

DRPR:

FIG. 3 shows the relative levels of Bcl-2 mRNA and .beta.2-microglobulin (control) mRNA in cells cultured for 4 hr and 19 hr at 37.degree. C. or 32.5.degree. C. Cell lines are as described in FIG. 2. The mRNA levels were determined using the reverse transcriptase-polymerase chain reaction method.

DEPR:

Bcl-2 is known to be involved in the process of apoptosis in a cell. Since p53 can induce cell death, the effect of the p53 tumor suppressor on Bcl-2 mRNA expression was determined using the reverse transcriptase-polymerase chain reaction (RT-PCR) method. As a control, the level of .beta.2-microglobulin mRNA was determined in each sample.

DEPR:

M1-p53 and M1-Neo were incubated at 32.5.degree. C. or 37.degree. C. for various times, then collected. In samples of M1-p53 cells harvested 19 hr after being shifted to 32.5.degree. C., dead cells were removed by centrifugation in Histopaque.TM. (SIGMA). Total RNA was isolated using TRIzol.TM. reagent (GIBCO/BRL) as described by the manufacturer. Reverse transcription was used to produce cDNA from the total RNA. Five .mu.g RNA was incubated in a 50 .mu.l reaction volume containing 200 U Moloney Leukemia Virus Reverse Transcriptase (GIBCO/BRL), 4 .mu.l random hexamer (62.5 A.sub.260 U/ml), 10 mM dithiothreitol, 20 U RNasin (Promega) and 1 mM each of dGTP, DATP, dTTP, dCTP in the reaction buffer provided by the manufacturer. Reactions were incubated at 37.degree. C. and allowed to proceed for 1 hr.

DEPR:

The cDNA product was amplified using the PCR method. Ten .mu.l of the cDNA product was resuspended in a 100 .mu.l reaction volume containing 2.5 U Taq DNA polymerase (Perkin-Elmer-Cetus), 60 mM each of dGTP, DATP, dTTP and dCTP and 50 pmol each of the forward and reverse primers in the reaction buffer provided by the manufacturer (primers are described below). Amplification cycles consisted of 94.degree. C. for 30 sec, 57.degree. C. for 30 sec, 72.degree. C. for 3 min. In order to obtain data within the linear range of the assay, each cDNA was amplified in serial cycles from 27 to 39 and 18 to 27 with increments of 3 cycles for bcl-2 and .beta.2-microglobulin, respectively.

DEPR:

Primers and internal probes used were as follows. Murine bcl-2 forward primer: 5'-TGACCTGAGCGCCTTCAC-3' (SEQ ID NO: 2); bcl-2 reverse primer: 5'-TAGCTGATTCGACCATTTGCCTGA-3' (SEQ ID NO: 3); bcl-2 internal probe: 5'-CCAGGAGAAATCAAACAAGG-3' (SEQ ID NO: 4); murine .beta.2-microglobulin forward primer: 5'ATGGCTCGCTCGGTGACCCTAG-3' (SEQ ID NO: 5); .beta.2-microglobulin reverse primer: 5'-TCATGATGCTTGATCACATGTCTCG-3' (SEQ ID NO: 6); .beta.2-microglobulin internal probe: 5'GCTACGTAACACAGTTCCAC-3' (SEQ ID NO: 7). Forward and reverse primers were designed to span exon-intron junctions and, therefore, do not amplify any genomic DNA that may contaminate the RNA samples. Expected sizes of the amplified products of bcl-2 and .beta.2-microglobulin were 575 bp and 373 bp, respectively.

DEPR:

M1-p53 and M1-Neo were incubated at 32.5.degree. C. or 37.degree. C. for various

times, then collected. In samples of M1-p53 cells harvested 19 hr after being shifted to 32.5 degree. C., dead cells were removed by centrifugation in Histopaque.TM. (SIGMA). Total RNA was isolated as described above and 15 .mu.g total RNA was size-fractionated in 1.2% agarose gels containing 2.2M formaldehyde (Sambrook et al., 1989). RNA was transferred to GeneScreen Plus.TM. nylon filters (NEN) using 10.times. SSC (1.times. SSC=0.15M NaCl/0.015M sodium citrate) and covalently bound to the membrane using UV irradiation. Probes, as described below, were labelled using .alpha.-³²P-dCTP by the random primer method (Sambrook et al., 1989; specific activity=1.times.10.⁹ cpm/.mu.g).

DEPR:

DEPR:
 .sup.32 P-labelled probes were added to the hybridization solution and hybridization was performed for 16 hr at 42.degree. C. (hybridization solution is 50% formamide, 10% dextran sulfate, 1M NaCl, 1% SDS, 1.times. Denhardt's solution, 25 mM Tris (pH 7.4) and 50 mg/ml denatured salmon sperm DNA) (50.times. Denhardt's solution contains 5 g Ficoll (Type 400; Pharmacia), 5 g polyvinylpyrrolidone, 5 g bovine serum albumin (Fraction V; Sigma) and water to 500 ml). Following hybridization, the filters were washed with 2.times. SSC/0.1% SDS at room temperature, then with the same solution at 68.degree. C. and exposed to X-ray film as described above.

DEPR:

DEPR:

A murine bax-specific probe was prepared by amplifying the entire open reading frame of the bax cDNA using RT-PCR as described above. The forward primer was 5'-GGAATTCGCGGTGATGGACGGGTCCGG-3' (SEQ ID NO: 8) and the reverse primer was 5'-GGAATTCTCAGCCCATCTTCTTCCAGA-3' (SEQ ID NO: 9). An Eco RI linker sequence was included at the 5'-end of each primer (underlined). Amplified cDNA products were gel-purified using GeneClean II.TM. (Bio 101, Inc.), cleaved using Eco RI (10 U/.mu.g DNA) and subcloned into a Bluescript plasmid pSK-II (Stratagene). An Eco RI restriction fragment of approximately 600 base pairs was excised from the cloned insert, gel-purified and used as a hybridization probe. The murine .beta.2-microglobulin cDNA probe is described by Parnes et al., Proc. Natl. Acad. Sci., USA 78:2253-2257 (1981), which is incorporated herein by reference.

DEPR:

DEPR: M1-p53 and M1-Neo were incubated at 32.5.degree. C. or 37.degree. C. for various times, then collected. In samples of M1-p53cells harvested 19 hr after being shifted to 32.5.degree. C., dead cells were removed by centrifugation in Histopaque.TM. (SIGMA). Cells were washed in ice cold phosphate-buffered saline (PBS; pH 7.4) and collected by centrifugation. The cell pellets were resuspended in ice-cold lysis buffer containing the protease inhibitors, 0.7 mg/ml pepstatin, 1 mM phenylmethylsulfonylfluoride (PMSF), 0.23 U/ml aprotinin, 10 mM leupeptin and 1 mM benzamidine (lysis buffer is 10 mM Tris (pH 7.4), 0.15M NaCl, 5 mM EDTA, 1% (v/v) Triton X-100). Following incubation on ice for 30 min, samples were centrifuged at 16,000.times.g for 10 min and the postnuclear supernatants were collected. Protein concentrations were determined using the bicinchoninic acid protein assay kit (Pierce, Inc.).

DEPR:

DEPR: Twenty μ g protein were size-fractionated under reducing conditions by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using 12% gels, as described by Laemmli et al. (Nature 227:680-685 (1970), which is incorporated herein by reference), then electrophoretically transferred to nitrocellulose filters. The filters were incubated for 2 hr at room temperature with preblocking solution (10 mM Tris (pH 7.6), 0.15M NaCl, 5% skim milk, 2% BSA and 0.1% Tween 20), then the solution was removed, fresh solution containing 0.1-0.2% (vol:vol) of the appropriate antibody (described below) was added and incubation was continued for 16 hr at 4.degree. C.

DEPR:

DEPR:

Following incubation with the first antibodies, the filters were washed 3.times. (5 min each) in a solution containing 0.12M NaCl, 8.7 mM NaH.sub.2 PO.sub.4, 31 mM K.sub.2 HPO.sub.4 (pH 7.6), then incubated with preblocking solution for 30 min, followed by fresh preblocking solution containing biotinylated goat anti-rabbit IgG (H+L) antibody (Vector Laboratories, Inc.). After washing as above, antibody binding was detected using an avidin-biotin-complex method, which employed the Vectastain ABC kit (Vector Laboratories Inc.) followed by the addition of 0.4 mg/ml AEC (3-amino-9-ethyl carbazole) containing 0.01% H.sub.2 O.sub.2 for color development.

DEPR:

The p53-RE.sup.D sequence was amplified with Pfu DNA polymerase (Stratagene) using the forward primer, 5'-GCGAAGCTTGTAGACTGATATTAAC-3' (SEQ ID NO: 12), and the reverse primer, 5'-GCGAAGCTTATAATCCAGCTATTTT-3' (SEQ ID NO: 13). A Hind III linker sequence was added to 5' end of each primer (underlined). Plasmid p18-21H was used as the template (Tsujimoto et al., Proc. Natl. Acad. Sci. USA 84:1329-1331 (1987), which is incorporated herein by reference).

DEPR:

The amplified p53-RE.sup.D product was gel-purified, then digested with Hind III and subcloned into the unique Hind III site of either pUCSV0CAT, which contains a chloramphenicol acetyltransferase (CAT) reporter gene, or pUCSV3CAT, which contains the CAT reporter gene and an SV40 promoter (Fukamizu et al., Biomed. Biochim. Acta 50:659-663 (1991), which is incorporated herein by reference). The Hind III site is located upstream of the CAT gene in pUCSV0CAT and between the SV40 promoter and the CAT gene in pUCSV3CAT; the constructed plasmids, therefore, were designated pUCSV40(p53-RE.sup.D) CAT and pUC(p53-RE.sup.D) CAT, respectively. Proper construction of the plasmids was confirmed by DNA sequencing.

DEPR:

The p53-RE.sup.D fragment also was subcloned in a position upstream or downstream of the SV40-CAT transcriptional unit in pUCSV3CAT. Essentially, the unique Hind III site was destroyed by digesting the plasmid with Hind III, then blunting the ends with the Klenow fragment of DNA polymerase I and self-ligating the plasmids. The BamHI site, which is located downstream of the CAT gene, and a Bgl II site, which is located upstream of the SV40 promoter then were converted to Hind III sites using the appropriate linkers. The p53-RE.sup.D fragment was subcloned into each of the newly created Hind III sites to generate the plasmids, pSV40CAT(p53-RE.sup.D) and p(p53-RE.sup.D)SV40CAT, respectively.

DEPR:

H358 cells were grown in T75 flasks and transfected with pCMV-p53.sub.wt or pCMV-p53.sub.179 as described above. Untransfected cells were used as a negative control. Flasks were washed 3.times. with Tris-buffered saline, then 2.5 ml lysis buffer was added to each flask (lysis buffer is 20 mM HEPES (pH 7.6), 20% glycerol, 10 mM NaCl, 1.5 mM MgCl.sub.2, 0.2 mM EDTA, 0.1% Triton X-100, 1 mM dithiothreitol (DTT), 1 mM PMSF, 10 .mu.g/ml leupeptin, 10 g/ml pepstatin and 100 .mu.g/ml aprotinin). Cells were dislodged by scraping and were pelleted by centrifugation at 2000 rpm at 4.degree. C. Nuclei were resuspended at 2.5.times.10.sup.7 nuclei per ml in nuclear extraction buffer, which is lysis buffer containing 500 mM NaCl. Nuclei were rocked gently for 1 hr at 4.degree. C., then centrifuged for 10 min at 10,000 rpm. The supernatant was aliquoted into cryotubes, then quick frozen in liquid nitrogen and stored at -80.degree. C.

DEPR:

Binding reactions were performed by adding 2 .mu.l poly dI-dC (1 mg/ml; Pharmacia), 16 .mu.l binding buffer, 1 l (.alpha.-.sup.32 P)dCTP-labelled p53-RE.sup.D probe (1.times.10.sup.4 cpm) and 1 .mu.l unlabelled competitor p53-RE.sup.D DNA (3 pmol) to 2 .mu.l of nuclear extract and incubating the reaction for 30 min at room temperature (binding buffer is 25 mM HEPES (pH 7.9), 0.5 mM EDTA (pH 8.0), 50 mM KCl, 10% glycerol, 0.5 mM DTT and 0.5 mM PMSF). In some experiments, 1 .mu.g of an anti-p53 monoclonal antibody was included in the reaction mixture.

DEPR:

The p53-RE.sup.D probe was isolated by Hind III digestion of pUCSV40(p53-RE.sup.D) CAT, followed by gel purification of the p53-RE.sup.D fragment. The probe was labelled using (.alpha.-.sup.32 P)dCTP and the Klenow fragment of DNA polymerase I (Sambrook et al., supra, 1989). Following the binding reaction, samples were subjected to polyacrylamide gel electrophoresis using a 4% gel and a buffer containing 50 mM Tris (pH 8.5), 0.4M glycine, 2 mM EDTA and 3% glycerol. The gels then were dried and exposed to X-ray film as described above.

DEPR:

Oligonucleotide primers for site-directed mutagenesis were 5'-GAAGATCTGAGACGGGGTTATCTCTT-3' (SEQ ID NO: 18; Bgl II site underlined); 5'-CGCGTCGACTGAGTGGTTTGTGTTTTT-3' (SEQ ID NO: 19; Sal I site underlined); 5'-

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AAGTTAGAGATAATGCTGGGCGTAGG-3' (SEQ ID NO: 20); and
5'-CCTACGCCCAGCATTATCTCTAACTT-3' (SEQ ID NO: 21; mutations are indicated in
bold). pTM604-4 was used as a template and amplification was performed using Pfu
heat-stable DNA polymerase (Stratagene, Inc.) as suggested by the manufacturer.
The PCR product was gel-purified, digested with Bgl II and Sal I and subcloned
into the Bgl II and Sal I sites of pA10-CATBS to produce pTM688-2. The presence
of the mutations was confirmed by DNA sequencing.

DEPR:

In vitro-translated wild-type and mutant p53.sup.179 proteins were prepared from
RNA generated using the T7 RNA polymerase binding sites in the plasmids
pCMV-p53.sub.wt and pCMV-p53.sub.179. Coupled in vitro transcription/translation
reactions were performed using T7 RNA polymerase and reticulocyte lysates
(TNT-Lysate.TM.; Promega, Inc.; Madison Wis.) as suggested by the manufacturer.
Oligomers A and D were annealed and filled-in and radiolabelled using Klenow
fragment in the presence of DATP, dGTP, TTP and .alpha.-.sup.32 P-dCTP.

DEPR:

Approximately 5 .mu.l of the p53 protein-containing translation product was
preincubated with no further additions or with the following monoclonal
antibodies: either a combination of 0.5 .mu.g anti-p53 IgG2.sub.a clone DO-1
(Santa Cruz Biotechnology, Inc.) and 0.5 .mu.g P421 (Oncogene Science, Inc.) or 1
.mu.g anti-CD2 control IgG.sub.2a antibody Leu-5b (Becton-Dickinson, Inc.; San
Jose Calif.). The reactions were incubated for 10 min at 25.degree. C. with 0.5
.mu.g sonicated salmon sperm DNA and 7 .mu.l EMSA buffer (20 mM HEPES, pH 7.5,
0.1M NaCl, 1.5 mM MgCl.sub.2, 10 mM dithiothreitol, 20% glycerol, 0.1% Triton
X100, 1 mM PMSF, 10 ug/ml pepstatin, 10 ug/ml leupeptin). The .sup.32 P-labelled
DNA probes (4.times.10.sup.5 cpm) were added and incubation was continued for 20
min at 25.degree. C. Following incubation, the samples were separated by
electrophoresis in non-denaturing 4% polyacrylamide gels using 1.times. TBE, then
the gels were dried and exposed to X-ray film (XAR; Kodak, Inc.) at -80.degree.
C. with intensifying screens.

WEST

Generate Collection

Search Results - Record(s) 51 through 60 of 95 returned.

☐ 51. Document ID: US 5910417 A

L16: Entry 51 of 95

File: USPT

Jun 8, 1999

US-PAT-NO: 5910417

DOCUMENT-IDENTIFIER: US 5910417 A

TITLE: Regulation of cytokine production in a hematopoietic cell

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 52. Document ID: US 5908750 A

L16: Entry 52 of 95

File: USPT

Jun 1, 1999

US-PAT-NO: 5908750

DOCUMENT-IDENTIFIER: US 5908750 A

TITLE: Screening assays for identifying agents that regulate the expression of genes involved in cell death

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 53. Document ID: US 5879910 A

L16: Entry 53 of 95

File: USPT

Mar 9, 1999

US-PAT-NO: 5879910

DOCUMENT-IDENTIFIER: US 5879910 A

TITLE: Hepatocyte growth factor protease domain variants

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 54. Document ID: US 5877403 A

L16: Entry 54 of 95

File: USPT

Mar 2, 1999

US-PAT-NO: 5877403

DOCUMENT-IDENTIFIER: US 5877403 A

TITLE: Papaya ringspot virus protease gene

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 55. Document ID: US 5863741 A

L16: Entry 55 of 95

File: USPT

Jan 26, 1999

US-PAT-NO: 5863741

DOCUMENT-IDENTIFIER: US 5863741 A

TITLE: Method for identifying inhibitors of cdc2 protein kinase from pneumocystis carinii

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 56. Document ID: US 5861498 A

L16: Entry 56 of 95

File: USPT

Jan 19, 1999

US-PAT-NO: 5861498

DOCUMENT-IDENTIFIER: US 5861498 A

TITLE: Nucleotides encoding immunophilin FKBP46 and fragments thereof

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 57. Document ID: US 5858702 A

L16: Entry 57 of 95

File: USPT

Jan 12, 1999

US-PAT-NO: 5858702

DOCUMENT-IDENTIFIER: US 5858702 A

TITLE: Isolation, cloning and expression of transmembrane water channel Aquaporin 5 (AQP5)

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 58. Document ID: US 5851984 A

L16: Entry 58 of 95

File: USPT

Dec 22, 1998

US-PAT-NO: 5851984

DOCUMENT-IDENTIFIER: US 5851984 A

TITLE: Method of enhancing proliferation or differentiation of hematopoietic stem cells using Wnt polypeptides

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 59. Document ID: US 5849479 A

L16: Entry 59 of 95

File: USPT

Dec 15, 1998

US-PAT-NO: 5849479

DOCUMENT-IDENTIFIER: US 5849479 A

TITLE: High-affinity oligonucleotide ligands to vascular endothelial growth factor (VEGF)

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 60. Document ID: US 5846713 A

L16: Entry 60 of 95

File: USPT

Dec 8, 1998

US-PAT-NO: 5846713

DOCUMENT-IDENTIFIER: US 5846713 A

TITLE: High affinity HKGF nucleic acid ligands and inhibitors

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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Generate Collection

Term	Documents
PHENOL.USPT.	109596
PHENOLS.USPT.	47322
CHOLOROFORM.USPT.	90
CHOLOROFORMS	0
(15 NOT (CHOLOROFORM OR PHENOL)).USPT.	95

Display

10

Documents, starting with Document:

61

Display Format:

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Generate Collection

Search Results - Record(s) 61 through 70 of 95 returned.

☐ 61. Document ID: US 5840295 A

L16: Entry 61 of 95

File: USPT

Nov 24, 1998

US-PAT-NO: 5840295

DOCUMENT-IDENTIFIER: US 5840295 A

TITLE: Nerve-derived transglutaminase enzyme

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 62. Document ID: US 5837834 A

L16: Entry 62 of 95

File: USPT

Nov 17, 1998

US-PAT-NO: 5837834

DOCUMENT-IDENTIFIER: US 5837834 A

TITLE: High affinity HKGF nucleic acid ligands and inhibitors

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 63. Document ID: US 5817457 A

L16: Entry 63 of 95

File: USPT

Oct 6, 1998

US-PAT-NO: 5817457

DOCUMENT-IDENTIFIER: US 5817457 A

TITLE: Methods and kits for detecting viral reverse transcriptase activity in a sample using an acidic pH or an elevated temperature

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 64. Document ID: US 5814479 A

L16: Entry 64 of 95

File: USPT

Sep 29, 1998

US-PAT-NO: 5814479

DOCUMENT-IDENTIFIER: US 5814479 A

TITLE: Bsk receptor-like tyrosine kinase

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 65. Document ID: US 5811098 A

L16: Entry 65 of 95

File: USPT

Sep 22, 1998

US-PAT-NO: 5811098

DOCUMENT-IDENTIFIER: US 5811098 A

TITLE: Antibodies to HER4, human receptor tyrosine kinase

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 66. Document ID: US 5811533 A

L16: Entry 66 of 95

File: USPT

Sep 22, 1998

US-PAT-NO: 5811533

DOCUMENT-IDENTIFIER: US 5811533 A

TITLE: High-affinity oligonucleotide ligands to vascular endothelial growth factor (VEGF)

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 67. Document ID: US 5792645 A

L16: Entry 67 of 95

File: USPT

Aug 11, 1998

US-PAT-NO: 5792645

DOCUMENT-IDENTIFIER: US 5792645 A

TITLE: Protein-polycation nucleic acid complexes and methods of use

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 68. Document ID: US 5770686 A

L16: Entry 68 of 95

File: USPT

Jun 23, 1998

US-PAT-NO: 5770686

DOCUMENT-IDENTIFIER: US 5770686 A

TITLE: ICAM-related protein fragments

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 69. Document ID: US 5760206 A

L16: Entry 69 of 95

File: USPT

Jun 2, 1998

US-PAT-NO: 5760206

DOCUMENT-IDENTIFIER: US 5760206 A

TITLE: Nucleotide sequence of soybean stearoyl-ACP desaturase gene

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 70. Document ID: US 5741671 A

L16: Entry 70 of 95

File: USPT

Apr 21, 1998

US-PAT-NO: 5741671

DOCUMENT-IDENTIFIER: US 5741671 A

TITLE: Isolation cloning and expression of transmembrane water channel
aquaporin 1(AQP1)

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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Term	Documents
PHENOL.USPT.	109596
PHENOLS.USPT.	47322
CHOLOROFORM.USPT.	90
CHOLOROFORMS	0
(15 NOT (CHOLOROFORM OR PHENOL)).USPT.	95

Display

10

Documents, starting with Document:

71

Display Format:

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WEST[Generate Collection](#)**Search Results - Record(s) 71 through 80 of 95 returned.**☐ **71. Document ID: US 5731424 A**

L16: Entry 71 of 95

File: USPT

Mar 24, 1998

US-PAT-NO: 5731424

DOCUMENT-IDENTIFIER: US 5731424 A

TITLE: High affinity TGF.beta. nucleic acid ligands and inhibitors

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ **72. Document ID: US 5731144 A**

L16: Entry 72 of 95

File: USPT

Mar 24, 1998

US-PAT-NO: 5731144

DOCUMENT-IDENTIFIER: US 5731144 A

TITLE: High affinity TGF.beta. nucleic acid ligands

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ **73. Document ID: US 5686592 A**

L16: Entry 73 of 95

File: USPT

Nov 11, 1997

US-PAT-NO: 5686592

DOCUMENT-IDENTIFIER: US 5686592 A

TITLE: High-affinity oligonucleotide ligands to immunoglobulin E (IgE)

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ **74. Document ID: US 5663293 A**

L16: Entry 74 of 95

File: USPT

Sep 2, 1997

US-PAT-NO: 5663293

DOCUMENT-IDENTIFIER: US 5663293 A

TITLE: ICAM-related protein

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 75. Document ID: US 5659024 A

L16: Entry 75 of 95

File: USPT

Aug 19, 1997

US-PAT-NO: 5659024

DOCUMENT-IDENTIFIER: US 5659024 A

TITLE: Promotors that regulate the expression of genes involved in cell death

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 76. Document ID: US 5656731 A

L16: Entry 76 of 95

File: USPT

Aug 12, 1997

US-PAT-NO: 5656731

DOCUMENT-IDENTIFIER: US 5656731 A

TITLE: Nucleic acid-amplified immunoassay probes

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 77. Document ID: US 5637471 A

L16: Entry 77 of 95

File: USPT

Jun 10, 1997

US-PAT-NO: 5637471

DOCUMENT-IDENTIFIER: US 5637471 A

TITLE: Therapeutic and diagnostic methods and compositions based on transducin-like enhancer of split proteins and nucleic acids

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 78. Document ID: US 5629155 A

L16: Entry 78 of 95

File: USPT

May 13, 1997

US-PAT-NO: 5629155

DOCUMENT-IDENTIFIER: US 5629155 A

TITLE: High-affinity oligonucleotide ligands to immunoglobulin E (IgE)

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 79. Document ID: US 5622828 A

L16: Entry 79 of 95

File: USPT

Apr 22, 1997

US-PAT-NO: 5622828

DOCUMENT-IDENTIFIER: US 5622828 A

TITLE: High-affinity oligonucleotide ligands to secretory phospholipase A2 (sPLA.sub.2)

K00C	Drawn Desc	Image
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Feb 11, 1997

TITLE: Dipeptidyl peptidase-I inhibitors and uses thereof

KMMC	Drawn Desc	Image
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Documents

109596

47322

90

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95

10

81

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WEST[Generate Collection](#)**Search Results - Record(s) 81 through 90 of 95 returned.**☐ 81. Document ID: US 5602003 A

L16: Entry 81 of 95

File: USPT

Feb 11, 1997

US-PAT-NO: 5602003

DOCUMENT-IDENTIFIER: US 5602003 A

TITLE: N-acetylglucosaminyltransferase V gene

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 82. Document ID: US 5599919 A

L16: Entry 82 of 95

File: USPT

Feb 4, 1997

US-PAT-NO: 5599919

DOCUMENT-IDENTIFIER: US 5599919 A

TITLE: Nucleic acid encoding a transiently-expressed kinetochore protein, and methods of use

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 83. Document ID: US 5580963 A

L16: Entry 83 of 95

File: USPT

Dec 3, 1996

US-PAT-NO: 5580963

DOCUMENT-IDENTIFIER: US 5580963 A

TITLE: Single-chain hepatocyte growth factor variants

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 84. Document ID: US 5547856 A

L16: Entry 84 of 95

File: USPT

Aug 20, 1996

US-PAT-NO: 5547856

DOCUMENT-IDENTIFIER: US 5547856 A

TITLE: Hepatocyte growth factor variants

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 85. Document ID: US 5541095 A

L16: Entry 85 of 95

File: USPT

Jul 30, 1996

US-PAT-NO: 5541095

DOCUMENT-IDENTIFIER: US 5541095 A

TITLE: Glycosaminoglycan specific sulfotransferases

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 86. Document ID: US 5514565 A

L16: Entry 86 of 95

File: USPT

May 7, 1996

US-PAT-NO: 5514565

DOCUMENT-IDENTIFIER: US 5514565 A

TITLE: Enzymatically-produced dimeric IL-2 and a nerve-derived transglutaminase enzyme for its preparation

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 87. Document ID: US 5484710 A

L16: Entry 87 of 95

File: USPT

Jan 16, 1996

US-PAT-NO: 5484710

DOCUMENT-IDENTIFIER: US 5484710 A

TITLE: Method of down-regulating a gene linked to a P-53 responsive element

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 88. Document ID: US 5443974 A

L16: Entry 88 of 95

File: USPT

Aug 22, 1995

US-PAT-NO: 5443974

DOCUMENT-IDENTIFIER: US 5443974 A

TITLE: Nucleotide sequence of soybean stearyl-ACP desaturase gene

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 89. Document ID: US 5424205 A

L16: Entry 89 of 95

File: USPT

Jun 13, 1995

US-PAT-NO: 5424205

DOCUMENT-IDENTIFIER: US 5424205 A

TITLE: Amyloidin protease and uses thereof

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 90. Document ID: US 5387676 A

L16: Entry 90 of 95

File: USPT

Feb 7, 1995

US-PAT-NO: 5387676

DOCUMENT-IDENTIFIER: US 5387676 A

TITLE: MN gene and protein

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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Term	Documents
PHENOL.USPT.	109596
PHENOLS.USPT.	47322
CHOLOROFORM.USPT.	90
CHOLOROFORMS	0
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91

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L16: Entry 91 of 95

File: USPT

Jan 24, 1995

US-PAT-NO: 5384250

DOCUMENT-IDENTIFIER: US 5384250 A

TITLE: Expression and purification of cloned alpha-fetoprotein

Full	Title	Citation	Front	Review	Classification	Date	Reference
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☐ 92. Document ID: US 5354844 A

L16: Entry 92 of 95

File: USPT

Oct 11, 1994

US-PAT-NO: 5354844

DOCUMENT-IDENTIFIER: US 5354844 A

TITLE: Protein-polycation conjugates

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 93. Document ID: US 5328837 A

L16: Entry 93 of 95

File: USPT

Jul 12, 1994

US-PAT-NO: 5328837

DOCUMENT-IDENTIFIER: US 5328837 A

TITLE: Hepatocyte growth factor protease domain variants

Full	Title	Citation	Front	Review	Classification	Date	Reference
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☐ 94. Document ID: US 5316921 A

L16: Entry 94 of 95

File: USPT

May 31, 1994

US-PAT-NO: 5316921

DOCUMENT-IDENTIFIER: US 5316921 A

TITLE: Single-chain hepatocyte growth factor variants

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 95. Document ID: US 5292652 A

L16: Entry 95 of 95

File: USPT

Mar 8, 1994

US-PAT-NO: 5292652

DOCUMENT-IDENTIFIER: US 5292652 A

TITLE: Amyloidin protease and uses thereof

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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CHOLOROFORMS	0
(15 NOT (CHOLOROFORM OR PHENOL)).USPT.	95

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May 21, 1991

TITLE: Reverse transcriptase and method for its production

One particular member of the pRT600 series, pRT601, used with the host E. coli strain N4830, is used for large-scale production of the M-MLV RT. A further feature of this invention is the method for purifying the enzyme from the large volume of medium which is needed to extract it from the cells. According to this method, the RT is precipitated with ammonium sulfate and re-solubilized, then further isolated by successive chromatographic adsorptions in the presence of non-ionic detergents; the process is described more fully below.

DEPR:

Frozen cells are thawed and a uniform suspension is made in an aqueous medium buffered to about pH 8.0 by the presence therein of Tris-HCl in a concentration of about 10 to 50 mM (optimally of 20 mM). The pH can vary in the range of about 7.3 to 8.4 which we will call mildly alkaline. Those skilled in the art will know that the actual working range will depend somewhat on experimental conditions but must be such that the protein is protected and has the proper adsorbency characteristics. A suitable reducing agent should be present in the medium. Such a reducing agent is dithiothreitol present in the medium at a concentration of from about 0.5 to 5 mM, optimally at 1.0 mM; others such as .alpha.-mercaptoethanol, will be known to those skilled in the art. It is preferred to include in the medium a chelator for heavy metals which deleteriously affect the active enzyme. A suitable such chelator is ethylenediamine - tetracetic acid (EDTA), present therein in a concentration of from about 0.1 to 2 mM, optimally 1 mM. It is also preferred to include a suitable inhibitor of proteolytic enzyme activity in order to preserve the stability of the enzyme. One such protease inhibitor is phenylmethylsulfonyl fluoride (PMSF), present in the medium at a concentration of from about 0.1 to 1 mM, optimally 0.2 mM. The medium must also contain an inert, soluble salt in order to preserve the enzyme in a soluble and active form. A suitable salt is NaCl, present in the medium at a concentration of from about 0.05 M to 0.15 M, optimally for extraction at 0.025 M. Where we have used NaCl in the various aqueous media, other monovalent salts such as KCl, NH₄Cl may usually be employed. After removal of nucleic acids, the optimum is 0.1 M. To the suspension is added a freshly prepared solution of lysozyme, preferably at about 13 mg/ml (0.1 ml of lysozyme/gm of starting cells) to digest cell walls. After thorough mixing, the suspension is allowed to stand for 20 minutes with occasional stirring.

A fraction containing the RT enzyme is eluted from the phosphocellulose with a linear salt gradient in medium buffered to pH 7.5 (i.e., 20 mM Tris-HCl) and containing 1 mM dithiothreitol, 1 mM EDTA, 5% (v/v) glycerol, 0.01% (w/v) nonionic detergent, and inert, soluble salt. The gradient runs from a low ionic strength of 0 to 0.15 M, optimally 0.1 M NaCl, to a high ionic strength of 0.4 to 0.8 M, preferably 0.5 M NaCl. The RT enzyme elutes as the major proteinaceous peak from the phosphocellulose column at 0.2 M to 0.3 M NaCl.

A fraction containing the RT enzyme is eluted from the heparin resin with a linear salt gradient in medium buffered to pH 7.5 (preferably 20 mM Tris-HCl) and containing 1 mM dithiothreitol, 1 mM EDTA, 5% (v/v) glycerol, 0.01% (w/v) nonionic detergent, and inert soluble salt. The gradient runs from a low ionic strength of 0.2 to 0.25 M to a high ionic strength of 0.5 to 1.5 M, optimally 1.0

M NaCl. The RT enzyme elutes as the only major proteinaceous peak from the heparin column at 0.2-0.25 M NaCl to 0.4 M NaCl.

DEPR:

Plasmid DNA was prepared from bacteria by the method of Birnboim and Doly (Birnboim, H. C. and Doly, J.; A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucl. Acids Res. 7 (1979) 1513-1523.). In some cases DNA was further purified by banding in CsCl-EtBr gradients (Maniatis et al., 1982). Nucleotide sequencing was performed by published methods (Maxam, A. M. and Gilbert, W.; Sequencing end-labeled DNA with base-specific chemical cleavages. Methods Enzymol 65 (1980) 499-560.) (Messing, J.; New M13 vectors for cloning. Methods Enzymol. 101C (1983) 20-89.). Electrophoresis of proteins was performed on 6 to 15% linear gradient polyacrylamide gels or 7% gels by the procedure of Laemmli (Laemmli, U. K.; Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. Nature 227 (1970) 680-685.). Unlabeled proteins were stained with Coomassie Blue (Grandgenett, D. P. et al., (1973). A single subunit from avian myeloblastosis virus with both RNA-directed DNA polymerase and ribonuclease H activity. Proc. Natl. Acad. Sci. USA 70, 230-234.) and [³⁵S]methionine-labeled proteins were detected by fluorography (Laskey, R. A.; the use of intensifying screens or organic scintillators for visualizing radioactive molecules resolved by gel electrophoresis. Methods Enzymol 65 (1980) 363-371.). To quantitate radio-labeled protein bands, exposed film was used as a template to excise regions of interest from gels. Gel pieces were extracted in organic scintillation fluid containing 3% Protosol (NEN) at 37 degree C. for 18 hours and counted. To quantitate stained protein bands, gels were scanned with an LKB Laser Densitometer equipped with a Hewlett Packard Integrator. Protein concentrations were determined by the method of Bradford (Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248-254.) with bovine serum albumin as standard.

DEPR:

RT activity in crude extracts was assayed utilizing poly(2'-0-methylcytidylate).oligodeoxyguanylate [(Cm).sub.n. (dG).sub.12-18] (Collaborative Research), a template-primer specific for RT and not copied by the DNA polymerases in E. coli (Gerard, G. F., et al., (1974) Poly (2'-0-methylcytidylate).oligodeoxyguanylate as template for the RNA directed DNA polymerase in RNA tumor virus particles and a specific probe for the RNA directed enzyme in transformed murine cells. Biochem. 13, 1632-1641.; Kotewicz, et al., 1985). Two .mu.l aliquots from extracts were incubated in 50 .mu.l reaction mixtures for 10 min at 37 C. Reaction mixtures contained 20 .mu.M Tris-HCl (pH 8.0), 1 mM dithiothreitol (DTT), 100 mM KCl, 2 mM MnCl₂, 50 .mu.M [³H]dGTP (150 cpm/pmol), 50 .mu.M (Cm).sub.n, and 20 .mu.M (dG).sub.12-18. Acid insoluble product was determined on Whatman GF/C glass fiber filters.

DEPR:

RT RNase H activity was assayed with [³H](A).sub.n. (dT).sub.n (Gerard, G. F. and Grandgenett, D. P. (1975). Purification and characterization of the DNA polymerase and RNase H activities in Moloney murine sarcoma-leukemia virus. J. Virol. 15, 785-797.). E. coli DNA polymerase I activity in extracts was assayed using (dA).sub.n.(dT).sub.12-18 as a primer template (Tamblyn, T. M. and Wells, R. D.; Comparative ability of RNA and DNA to prime DNA synthesis in vitro; role of sequence, sugar, and structure of template-primer. Biochemistry 14 (1975) 1412-1425.).

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L11: Entry 2 of 5

File: USPT

Aug 10, 1999

DOCUMENT-IDENTIFIER: US 5935803 A

TITLE: Methods to identify immunomodulators using cognate interaction of PKC-theta

DEPR:

In addition, the "two-hybrid" system may be used to effect interaction between a PKC or a fragment thereof and its cognate, and the effect of a candidate on this interaction can be observed. The "two-hybrid" system is described in U.S. Pat. No. 5,283,173, incorporated herein by reference. Briefly, as applied to the present invention, a recombinant host, typically yeast, is transformed with two expression systems each encoding a fusion protein. One fusion protein contains a portion of a transcription-activating factor fused to a PKC or cognate-binding fragment thereof, the other fusion protein contains the complementary portion of the transcription-activating portion fused to the cognate. Typically, the transcription-activating factor is an activator for RNA polymerase, and one portion represents the DNA-binding portion, the other the activator for the polymerase. When the cognate and PKC bind, the two portions of the transcription factor are brought into sufficient proximity that they are able to perform the function of activating transcription. The "two-hybrid" assay thus, also, will include a reporter expression system which is activated by the completed transcription factor to produce a reporter protein, such as .beta.-galactosidase or chloramphenicol acetyl transferase. As defined herein, "two-hybrid assay" refers to this general approach.

DEPR:

In addition to the pharmacologically active agent, a composition comprising an agent of the present invention may contain suitable pharmaceutically acceptable carriers such as excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically for delivery to the site of action. Suitable formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form, for example, water-soluble salts. In addition, suspensions of the active compounds as appropriate oily injection suspensions may be administered. Suitable lipophilic solvents or vehicles include fatty oils, for example, sesame oil, or synthetic fatty acid esters, for example, ethyl oleate or triglycerides. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension include, for example, sodium carboxymethyl cellulose, sorbitol, and/or dextran. Optionally, the suspension may also contain stabilizers. Liposomes can also be used to encapsulate the agent for delivery into the cell.

DEPR:

To identify the physiologically relevant cognate binding partner, a Triton (non-ionic detergent) cell extract was prepared from Jurkat T-cells (a human T-cell lymphoma line) using standard procedures. Based on the prior experience that physiologically relevant cognate binding partners for PKC may be associated with the particulate fraction, the "Triton extract" included both soluble and some particulate fraction proteins and is referred to herein as the Triton extract. A V1-his tail construct was also engineered; six histidine residues were attached to the N-terminus of V1. The six histidine residues bind to nickel agarose affinity beads.

DEPR:

FIG. 8 thus shows the results of the two-hybrid assay wherein the fyn peptide to be tested is fused to a DNA encoding the polymerase activating domains (of the VP 16 transcription factor) and the relevant portion of the PKC-theta protein is fused to the DNA binding region of the transcription factor (LexA). Beta

galactosidase was used as a reporter gene for the results in this Figure.

DEPR:

In this approach, plasmids containing the polymerase activating domain fused to the kinase or regulatory domains of fyn were mixed with similar vectors containing a cDNA library from the murine T-cell line HT2 in a ratio of 1:500. Yeast harboring plasmids containing PKC-theta V1 fused to the DNA binding domain were transformed with this mixture of plasmids at one-tenth of the regular protocol. The yeast were plated onto media selecting for presence of the plasmids (THULL) plates and assayed for .beta.-galactosidase on filter lifts. Positive colonies were picked on THULL grids and retested. DNA was extracted from colonies which remained positive, and then amplified using 5' and 3' plasmid-based polylinker flanking primers and the products were analyzed by Southern blot using fyn as a probe.

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